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(57) Abstract

Probe sequences that are useful for detecting oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are herein provided. These sequences can be used in hybridization assays or amplification based assays designed to detect the presence of these oncogenic HPV types in a test sample. Additionally, the sequences can be provided as part of a kit.

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NUCLEIC ACID PRIMERS AND PROBES FOR DETECTING ONCOGENIC HUMAN PAPILLOMAVIRUSES

Field of the Invention

The present invention relates to human papillomaviruses and, in particular, it relates to oligonucleotides for detecting human papillomaviruses in a test sample.

Background of the Invention

To date, approximately seventy different human papillomavirus (HPV) types have been discovered. HPV is interesting from a diagnostic standpoint because several of the presently known HPV types have been linked to the development of cervical cancer. As with any form of cancer, early detection is critical to successfully treating the disease. Because certain HPV strains are associated with the development of cervical cancer, detecting HPV in an appropriate sample may provide the best means for the early detection of cervical cancer.

The polymerase chain reaction in combination with Southern blot analysis has been the prevailing method for detecting particular types of HPV in a test sample. In particular Snijders, P.J.F., et. al., J. of Gen. Virol., Vol. 71, pp.173-181 (1990) exemplifies such technology where amplification primers are employed to generate multiple copies of a sequence within the HPV genome and radiolabeled DNA probes specific for a particular HPV type are employed to detect and thereby determine the particular HPV type present in the test sample. Unfortunately, Southern blotting is a relatively labor intensive and time consuming process especially when attempting to detect multiple different HPV types. Accordingly, there is a need for methods and reagents suitable for quickly and accurately determining whether or not one or several of the HPV types associated with cervical cancer are present in a test sample.

Summary of the Invention

The present invention provides oligonucleotides that can be used to specifically detect oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 (hereinafter "oncogenic HPV types"). These oligonucleotides are designated SEQ ID NO 4 and its complement SEQ ID NO 5; SEQ ID NO 7 and its complement SEQ ID NO 8; SEQ ID NO 10 and its complement SEQ ID NO 11; SEQ ID NO 13 and its complement SEQ ID NO 14; SEQ ID NO 16 and its complement SEQ ID NO 17; SEQ ID NO 19 and its complement SEQ ID NO 20; SEQ ID NO 22 and its complement SEQ ID NO 23; SEQ ID NO 25 and its complement SEQ ID NO 26; SEQ ID NO 28 and its complement SEQ ID NO 29; SEQ ID NO 31 and its complement SEQ ID NO 32; SEQ ID NO 34 and its complement SEQ ID NO 35; SEQ ID NO 37 and its complement SEQ ID NO 38; as well as SEQ ID NO 40 and its complement SEQ ID NO 41. Preferred are cocktails of these probes comprising two or more of the above oligonucleotides.

Preferably, the oligonucleotides are employed as hybridization probes to hybridize with and detect target sequences for which they are specific. Thus, methods provided by the present invention include hybridization assays as well as amplification based assays. According to one method, a method of detecting the presence of at least one oncogenic HPV type in a test sample comprises the steps of (a) contacting the test sample with one or more of the sequences listed above; and (b) detecting hybridization between at least one of the above sequences and an oncogenic HPV target sequence as an indication of the presence of at least one oncogenic HPV type in the test sample.

According to another embodiment, a method for detecting the presence of at least one oncogenic HPV type in a test sample comprises the steps of (a) forming a reaction mixture comprising nucleic acid amplification reagents, a test sample containing an oncogenic HPV target sequence, at least one (and preferably two) primer(s) capable of amplifying an HPV target sequence designated herein as SEQ ID NO. 3, SEQ ID NO. 6, SEQ ID NO. 9, SEQ ID NO. 12, SEQ ID NO. 15, SEQ ID NO. 18, SEQ ID NO. 21, SEQ ID NO. 24, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 33, SEQ ID NO. 36, and SEQ ID NO. 39 and one or more oligonucleotides selected from the group consisting of SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO. 10, SEQ ID NO 13, SEQ ID NO 16, SEQ ID NO 19, SEQ ID

NO 22, SEQ ID NO 25, SEQ ID NO 28, SEQ ID NO 31, SEQ ID NO 34, SEQ ID NO 37, SEQ ID NO 40, and their respective complements; (b) subjecting the mixture to hybridization conditions to produce at least one nucleic acid sequence complementary to the target sequence; (c) hybridizing one or more oligonucleotides to the nucleic acid sequence complementary to the target sequence, so as to form at least one complex comprising the oligonucleotide and the complementary nucleic acid sequence; and (d) detecting the so-formed complex as an indication of the presence of at least one oncogenic HPV type in the sample.

According to another embodiment, the invention provides kits which comprise a set of oligonucleotide primers, amplification reagents and at least one, and preferably at least two, of the oligonucleotides designated as SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 40 and SEQ ID NO. 41.

Detailed Description of the Invention

As previously mentioned, the present invention provides oligonucleotides (hereinafter "oligos" or "probes"), methods for using the probes and kits containing the probes, all of which can be employed to specifically detect oncogenic HPV types (i.e. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The probes provided herein can be employed as primers in an amplification reaction but preferably are employed as hybridization probes because each of the probes is specific for at least one HPV type and in one case (SEQ ID NO. 34) two HPV types. Advantageously, all of the probes hybridize within an approximately 140 bp region of the L1 gene found in the HPV genome. Thus, while the probes individually can be used to detect the oncogenic HPV type(s) for which they are specific, a cocktail comprising two or more of the oligos can be employed to detect several HPV types at once. This is particularly advantageous in an amplification reaction setting where all, more or part of the approximately 140 bp region can be amplified and the amplified product can be contacted with a cocktail of probes to determine the presence of at least one of the oncogenic HPV

types in the test sample. Accordingly, a single amplification reaction can be the basis for detecting multiple HPV types. Table 1 below provides the SEQ ID NOs. of the oligos provided herein, the sequences and the HPV type(s) that they specifically detect.

SEQ ID NO.	SEQUENCE 5' -> 3'	HPV TYPE SPECIFICITY
4	GCTGCCATAT CTACTTCA	16
5	TGAAGTAGAT ATGGCAGC	16
7	GTAGCATCAT ATTGCC	18
8	GGCAATATGA TGCTAC	18
10	GCAATTGCAA ACAGTGAT	31
11	ATCACTGTTT GCAATTGC	31
13	ATGCACACAA GTAACTAGT	33
14	ACTAGTTACT TGTGTGCAT	33
16	CTGCTGTGTC TTCTAGTG	35
17	CACTAGAAGA CACAGCAG	35
19	CTCTATAGAG TCTTCCATAC C	39
20	GGTATGGAAG ACTCTATAGA G	39
22	CTACACAAAA TCCTGTG	45
23	CACAGGATTT TGTGTAG	45
25	CGGTTTCCCC AACAT	51
26	ATGTTGGGGA AACCG	51
28	GTGCTGAGGT TAAAAAG	52
29	CTTTTTAACC TCAGCAC	52
31	CTACAGAACA GTTAAGTAA	56
32	TTACTTAACT GTTCTGTAG	56
34	AACTAAGGAA GGTACAT	58/33
35	ATGTACCTTC CTTAGTT	58/33
37	CTACTACTCT CTATTCCTAA TG	59
38	CATTAGGAAT AGAGAGTAGT AG	59
40	CTTTGTCTAC TACTACTGA	68
41	TCAGTAGTAG TAGACAAAG	68

The probes disclosed herein may comprise deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or nucleic acid analogs such as uncharged nucleic acid analogs including but not limited to peptide nucleic acids (PNAs) which are disclosed in International Patent Application WO 92/20702 or morpholino analogs which are described in U.S. Patents Numbered 5,185,444, 5,034,506, and 5,142,047 all of which are herein incorporated by reference. Such sequences can routinely be synthesized using a variety of techniques currently available. For example, a sequence of DNA can be synthesized using conventional nucleotide phosphoramidite chemistry and the instruments available from Applied Biosystems, Inc, (Foster City, CA); DuPont, (Wilmington, DE); or Milligen, (Bedford, MA). Similarly, and when desirable, the sequences can be labeled using methodologies well known in the art such as described in U.S. Patent Applications Numbered 5,464,746; 5,424,414; and 4,948,882 all of which are herein incorporated by reference.

The term "label" as used herein means a molecule or moiety having a property or characteristic which is capable of detection. A label can be directly detectable, as with, for example, radioisotopes, fluorophores, chemiluminophores, enzymes, colloidal particles, fluorescent microparticles and the like; or a label may be indirectly detectable, as with, for example, specific binding members. It will be understood that directly detectable labels may require additional components such as, for example, substrates, triggering reagents, light, and the like to enable detection of the label. When indirectly detectable labels are used, they are typically used in combination with a "conjugate". A conjugate is typically a specific binding member which has been attached or coupled to a directly detectable label. Coupling chemistries for synthesizing a conjugate are well known in the art and can include, for example, any chemical means and/or physical means that does not destroy the specific binding property of the specific binding member or the detectable property of the label. As used herein, "specific binding member" means a member of a binding pair, i.e., two different molecules where one of the molecules through, for example, chemical or physical means specifically binds to the other molecule. In addition to antigen and antibody specific binding pairs, other specific binding pairs include, but are not intended to be limited to, avidin and biotin; haptens and antibodies specific for haptens; complementary

nucleotide sequences; enzyme cofactors or substrates and enzymes; and the like.

Generally, the probes provided herein can be employed to detect the presence of an oncogenic HPV type in a test sample by contacting a test sample with at least one of the sequences provided herein under hybridizing conditions, and detecting hybridization between an HPV target sequence and at least one of the sequences designated herein as SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 40 and SEQ ID NO 41. Several well known methods for detecting hybridization can be employed according to the present invention and may include, for example, the use of gels and stains or detecting a label associated with one or more of the sequences provided herein after performing, for example, a dot blot or amplification reaction.

The term "test sample" as used herein, means anything suspected of containing a target sequence. The test sample can be derived from any biological source and can be used (i) directly as obtained from the source or (ii) following a pre-treatment to modify the character of the sample. Thus, the test sample can be pre-treated prior to use by, for example, disrupting cells, preparing liquids from solid materials, diluting viscous fluids, filtering liquids, distilling liquids, concentrating liquids, inactivating interfering components, adding reagents, purifying nucleic acids, and the like. Typically, the test sample will be or be derived from cervical scrapes or similar samples.

A "target sequence" as used herein means a nucleic acid sequence that is detected, amplified, both amplified and detected or otherwise is complementary to one of the probes herein provided. Additionally, while the term target sequence is sometimes referred to as single stranded, those skilled in the art will recognize that the target sequence may actually be double stranded.

"Hybridization" or "hybridizing" conditions are defined generally as conditions which promote annealing between complementary nucleic acid sequences or annealing and extension of one or more nucleic acid sequences. It is well known in the art that such annealing is dependent

in a rather predictable manner on several parameters, including temperature, ionic strength, sequence length, complementarity, and G:C content of the sequences. For example, lowering the temperature in the environment of complementary nucleic acid sequences promotes annealing. For any given set of sequences, melt temperature, or Tm, can be estimated by any of several known methods. Typically, diagnostic applications utilize hybridization temperatures which are close to (i.e. within 10°C) the melt temperature. Ionic strength or "salt" concentration also impacts the melt temperature, since small cations tend to stabilize the formation of duplexes by negating the negative charge on the phosphodiester backbone. Typical salt concentrations depend on the nature and valency of the cation but are readily understood by those skilled in the art. Similarly, high G:C content and increased sequence length are also known to stabilize duplex formation because G:C pairings involve 3 hydrogen bonds where A:T pairs have just two, and because longer sequences have more hydrogen bonds holding the sequences together. Thus, a high G:C content and longer sequence lengths impact the hybridization conditions by elevating the melt temperature.

Once sequences are selected for a given diagnostic application, the G:C content and length will be known and can be accounted for in determining precisely what hybridization conditions will encompass. Since ionic strength is typically optimized for enzymatic activity, the only parameter left to vary is the temperature. Generally, the hybridization temperature is selected close to or at the Tm of the primers or probe. Thus, obtaining suitable hybridization conditions for a particular primer, probe, or primer and probe set is well within ordinary skill of one practicing this art.

The sequences provided herein also can be used as amplification primers according to amplification procedures well known in the art. Such reactions include, but are not intended to be limited to, the polymerase chain reaction (PCR) described in U.S. Patents 4,683,195 and 4,683,202, the ligase chain reaction (LCR) described in EP-A-320 308, and gap LCR (GLCR) described in U.S. Patent No. 5,427,930 all of which are herein incorporated by reference.

According to a preferred embodiment, the probes are employed in the "oligonucleotide hybridization PCR" (variably referred to herein as

"OH PCR") amplification reaction as described in U.S. Patent Application Serial No. 08/514,704, filed August 14, 1995, that is herein incorporated by reference. Briefly, the reagents employed in the preferred method comprise at least one amplification primer (preferably two) and at least one probe, as well as other reagents for performing an amplification reaction.

The primer sequence is employed to prime extension of a copy of a target sequence (or its complement) and is labeled with either a capture label or a detection label. The probe sequence is used to hybridize with the sequence generated by the primer sequence, and typically hybridizes with a sequence that does not include the primer sequence or its exact complement. Similarly to the primer sequence, the probe sequence is also labeled with either a capture label or a detection label with the caveat that when the primer is labeled with a capture label, the probe is labeled with a detection label and vice versa. Detection labels have the same definition as "labels" previously defined and "capture labels" are typically used to separate extension products, and probes associated with any such products, from other amplification reactants. Specific binding members (as previously defined) are well suited for this purpose. Also, probes used according to the OH PCR method are preferably blocked at their 3' ends so that they are not extended under hybridization conditions. Methods for preventing extension of a probe are well known and are a matter of choice for one skilled in the art. For example, adding a phosphate group or label to the 3' end of the probe generally will suffice for purposes of blocking extension of the probe.

"Other reagents for performing an amplification reaction" or "nucleic acid amplification reagents" include reagents which are well known and may include, but are not limited to, an enzyme having polymerase activity, enzyme cofactors such as magnesium; salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates (dNTPs) such as for example deoxyadenine triphosphate, deoxyguanine triphosphate, deoxycytosine triphosphate and deoxythymine triphosphate.

The OH PCR method generally comprises the steps of (a) forming a reaction mixture comprising nucleic acid amplification reagents, one or more probes herein provided, at least one amplification primer and a

test sample suspected of containing a target sequence; (b) subjecting the mixture to hybridization conditions to generate at least one copy of a nucleic acid sequence complementary to the target sequence; (c) hybridizing the probe to the nucleic acid sequence complementary to the target sequence, so as to form a hybrid comprising the probe and the nucleic acid sequence complementary to the target sequence; and (d) detecting the hybrid as an indication of the presence of at least one oncogenic HPV type in the sample. It will be understood that step (b) of the above method can be repeated several times prior to step (c), by thermal cycling the reaction mixture as is well known in the art.

According to the above method, it is preferable to select primers, probes and reaction conditions such that the probe sequence has a lower melt temperature than the primer sequences so that upon placing the reaction mixture under hybridization conditions copies of the target sequence or its complement are produced at a temperature above the Tm of the probe. After such copies are synthesized, they are denatured and the mixture is cooled to enable the formation of hybrids between the probes and any copies of the target or its complement. The rate of temperature reduction from the denaturation temperature down to a temperature at which the probes will bind to single stranded copies is preferably quite rapid (for example 8 to 15 minutes) and particularly through the temperature range in which an enzyme having polymerase activity is active for primer extension. Such a rapid cooling favors copy sequence/probe hybridization rather that primer/copy sequence hybridization and extension.

Upon formation of the copy sequence/probe hybrids, the differential labels (i.e. capture and detection labels) on the copy sequence and probe can be used to separate and detect such hybrids. Preferably, detection is performed according to the protocols used by the commercially available Abbott LCx® instrumentation (Abbott Laboratories; Abbott Park, IL).

Thus, keeping the preferred method in mind, preferred reaction mixtures include one or more of the probes of the present invention and a primer or set of primers that prime extension of copies of target sequences having SEQ ID NOs. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, and 39 (i.e. at least one primer sequence and at least one probe sequence complementary to the extension product of the primer). SEQ ID NO. 1

and/or SEQ ID NO. 2 are exemplary sequences suitable for generating copies of the target sequences to which the probes of the present invention hybridize.

As previously mentioned, according to another embodiment, a cocktail comprising two or more of the probes are employed to detect whether at least one oncogenic HPV type is present in a test sample. Most preferably, the cocktail is a component of an amplification reaction mixture where all of the probes of the present invention are part of the cocktail. The probes are hybridized with the amplification products to form complexes and any complexes are then detected as an indication of whether at least one oncogenic HPV type was present in the test sample. Most preferably, cocktails according to the present invention will contain at least SEQ ID NO 37 or its complement SEQ ID NO 38, and SEQ ID NO 40 or its complement SEQ ID NO 41.

The probes of the present invention can be provided as part of a kit useful for detecting the presence of at least one oncogenic HPV type in a test sample. The kits comprise one or more suitable containers containing one or more sequences according to the present invention, an enzyme having polymerase activity, and deoxynucleotide triphosphates. Typically, at least one sequence bears a label, but detection is possible without this.

The following examples are provided to further illustrate the present invention and not intended to limit the invention.

Examples

The following examples demonstrate detection of oncogenic strains of human papillomaviruses (HPV) using primers to amplify the target sequences and the probes herein provided. These DNA primers and probes are identified as SEQUENCE ID NO. 1, SEQUENCE ID NO. 2, SEQUENCE ID NO. 4, SEQUENCE ID NO. 7, SEQUENCE ID NO. 10, SEQUENCE ID NO. 13, SEQUENCE ID NO. 16, SEQUENCE ID NO. 19, SEQUENCE ID NO. 22, SEQUENCE ID NO. 25, SEQUENCE ID NO. 28, and SEQUENCE ID NO. 31, SEQUENCE ID NO. 34, SEQUENCE ID NO. 37 and SEQUENCE ID NO. 40. All the above primers and probes are specific for a region in the L1 gene of HPV. In the following examples SEQUENCE ID NOs. 1 and 2 are used as consensus amplification primers specific for this region in oncogenic

and non-oncogenic types of HPV. A portion of the L1 sequence in oncogenic HPV Type 16 is designated herein as SEQ ID NO. 3. SEQ ID NO. 4 is used as a type-specific internal hybridization probe for oncogenic HPV Type 16. A portion of the L1 sequence in oncogenic HPV Type 18 is designated herein as SEQ ID NO. 6. SEQ ID NO. 7 is used as a type-specific internal hybridization probe for oncogenic HPV Type 18. A portion of the L1 sequence in oncogenic HPV Type 31 is designated herein as SEQ ID NO. 9. SEQ ID NO. 10 is used as a type-specific internal hybridization probe for oncogenic HPV Type 31. A portion of the L1 sequence in oncogenic HPV Type 33 is designated herein as SEQ ID NO. 12. SEQ ID NO. 13 is used as a type-specific internal hybridization probe for oncogenic HPV Type 33. A portion of the L1 sequence in oncogenic HPV Type 35 is designated herein as SEQ ID NO. 15. SEQ ID NO. 16 is used as a type-specific internal hybridization probe for oncogenic HPV Type 35. A portion of the L1 sequence in oncogenic HPV Type 39 is designated herein as SEQ ID NO. 18. SEQ ID NO. 19 is used as a type-specific internal hybridization probe for oncogenic HPV Type 39. A portion of the L1 sequence in oncogenic HPV Type 45 is designated herein as SEQ ID NO. 21. SEQ ID NO. 22 is used as a type-specific internal hybridization probe for oncogenic HPV Type 45. A portion of the L1 sequence in oncogenic HPV Type 51 is designated herein as SEQ ID NO. 24. SEQ ID NO. 25 is used as a type-specific internal hybridization probe for oncogenic HPV Type 51. A portion of the L1 sequence in oncogenic HPV Type 52 is designated herein as SEQ ID NO. 27. SEQ ID NO. 28 is used as a type-specific internal hybridization probe for oncogenic HPV Type 52. A portion of the L1 sequence in oncogenic HPV Type 56 is designated herein as SEQ ID NO. 30. SEQ ID NO. 31 is used as a type-specific internal hybridization probe for oncogenic HPV Type 56. A portion of the L1 sequence in oncogenic HPV Type 58 is designated herein as SEQ ID NO. 33. SEQ ID NO. 34 is used as a type-specific internal hybridization probe for oncogenic HPV Type 58. A portion of the L1 sequence in oncogenic HPV Type 59 is designated herein as SEQ ID NO. 36. SEQ ID NO. 37 is used as a type-specific internal hybridization probe for oncogenic HPV Type 59. A portion of the L1 sequence in oncogenic HPV Type 68 is designated herein as SEQ ID NO. 39. SEQ ID NO. 40 is used as a type-specific internal hybridization probe for oncogenic HPV Type 68.

Example 1
Preparation of HPV Primers and Probes

A. L1 Consensus Primers Target-specific consensus primers were designed to detect the HPV L1 target sequence of oncogenic and non-oncogenic HPV types by oligonucleotide hybridization PCR. These primers were SEQ ID NO. 1 and SEQ ID NO. 2. Primer sequences were synthesized using standard oligonucleotide synthesis methodology and haptenated with adamantane at their 5' ends using standard cyanoethyl phosphoramidite coupling chemistry as described in U.S. Patent No. 5,424,414 incorporated herein by reference.

B. L1 HPV Type-specific Probes The detection probes were designed to hybridize with the amplified HPV L1 target sequence by oligonucleotide hybridization. These probes are SEQ ID NO. 4 for HPV Type 16, SEQ ID NO. 7 for HPV Type 18, SEQ ID NO. 10 for HPV Type 31, SEQ ID NO. 13 for HPV Type 33, SEQ ID NO. 16 for HPV Type 35, SEQ ID NO. 19 for HPV Type 39, SEQ ID NO. 22 for HPV Type 45, SEQ ID NO. 25 for HPV Type 51, SEQ ID NO. 28 for HPV Type 52, SEQ ID NO. 31 for HPV Type 56, SEQ ID NO. 34 for HPV Type 58, SEQ ID NO. 37 for HPV Type 59 and SEQ ID NO. 40 for HPV Type 68. Probe SEQ ID NOs. 7, 10, 22, 25, 28, 31, 34, 37 and 40 were synthesized using standard oligonucleotide synthesis methodology and haptenated with 2 carbazoles at the 5' end using standard cyanoethyl phosphoramidite coupling chemistry as described in U.S. Patent No. 5,464,746 (herein incorporated by reference), and blocked with phosphate at the 3' end. Probe SEQ ID NOs. 4, 13, 16 and 19 were synthesized using standard oligonucleotide synthesis methodology and haptenated with 2 carbazoles at the 3' end using standard cyanoethyl phosphoramidite coupling chemistry as described in U.S. Patent No. 5,464,746. Reactivity was assessed against a known standard of HPV DNA.

Example 2
Sensitivity of HPV Detection

Plasmids, individually comprising the13 oncogenic HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) were prepared using the Qiagen Plasmid Kit (Qiagen Inc., Chatsworth, CA) according to the manufacturers instructions. In particular, for each plasmid, individual bacterial cultures containing the plasmids were grown overnight in 500 ml of TB media (1.2% bacto-trypton, 2.4% bacto-yeast extract, 0.4% v/v glycerol, 17 mM KH_2PO_4, 72 mM K_2HPO_4, 50 µg/ml ampicilin). The cells were collected by centrifugation (4°C, 10 minutes at 6000 x g) and the pellet was resuspended in 10 ml of 50 mM Tris-HCl, 50 mM EDTA, 100 µg/ml RNase A (pH 8.0). 10 ml of 0.2 N NaOH, 1% SDS was then added to the resuspended pellet and the resulting solution was incubated at room temperature for 5 minutes. After the incubation period, 10 ml of 3 M KAc (pH 5.5) was added to the solution and this solution was then incubated on ice for 20 minutes. After incubation, cellular debris was removed from the mixtures by centrifugation (4°C, 30 minutes at 15,000 x g) and the resulting supernatants were loaded onto a QIAGEN-tip 500 (Qiagen Inc.), equilibrated with 10 ml of 750 mM NaCl, 50 mM MOPS, 15% ethanol, 0.15% Triton® X-100 (pH 7.0). The QIAGEN-tip 500 was washed twice with 1.0 M NaCl, 50 mM MOPS, 15% ethanol (pH 7.0) before the DNA was eluted with 1.25 M NaCl, 50 mM MOPS, 15% ethanol, 0.15% Triton® X-100 (pH 8.5). DNA was precipitated from the eluant with 0.7 volumes of isopropanol and recovered by centrifugation (4°C, 30 minutes at15,000 x g). The DNA pellets were washed with 15 ml of cold 70% ethanol, air dried for 5 minutes and dissolved in 500 µl TE buffer (10 mM tris(hydroxymethyl) aminomethane (Tris®), 1 mM ethylenediamene tetraacetic acid (EDTA), pH 8.0)

The HPV plasmids were quantitated by comparison to a known DNA standard (linear M13 rf DNA from New England Biolabs, Beverly, MA) using agarose gel electrophoresis. To accomplish this the SYBR Green 1 (Molecular Probes, Eugene OR) fluorescence of the standards and HPV samples was measured with an IS-1000 digital imaging system (Alpha Innotech, San leandro CA) and the HPV plasmid concentration calculated from the M13 standard curve.

Separate dilution sets of the purified HPV DNAs were PCR amplified and detected using the HPV consensus primers (SEQ ID NOs. 1 and 2) and the HPV detection probes (SEQ ID NOs. 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37 and 40 for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, respectively) described in Example 1. PCR extension was performed using 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.5 mM EDTA, 10 µg/ml Bovine Serum Albumin (BSA) and 0.04% NaN_3. Taq polymerase was used at a concentration of 3.75 units and nucleotides were added giving a final concentration of 0.4 mM each. Primers were used at a concentration of 0.5 µM each, with probes for HPV types 51, 52, 56, 58 and 68 used at 3 µM, for HPV types 18 and 31 used at 4.5 µM, for HPV type 59 used at 6 µM and for HPV types 16, 33, 35, 39 and 45 used at 12 µM. A final concentration of 7 mM $MgCl_2$ was added at the same time as the sample. Testing was done using 50 µl of sample in a total reaction volume of 0.2 ml, with samples tested in duplicate using salmon testis DNA as a negative control.

Reaction mixtures were amplified in a Perkin-Elmer 480 Thermal Cycler. The following cycling conditions were used: 94°C for 2 minutes followed by cycling at 95°C for 20 seconds/44°C for 1.5 minutes/72°C for 1 minute for 5 cycles, then 95°C for 5 seconds/54°C for 30 seconds/72°C for 15 seconds for 40 cycles, then 72°C for 4 minutes. After the reaction mixtures were thermal cycled, the mixtures were maintained at 97°C for 2 minutes and probe oligo hybridization was accomplished by rapidly lowering the temperature to 4°C.

After the reaction products reached 4°C they were detected on the Abbott LCx® system (available from Abbott Laboratories, Abbott Park, IL). A suspension of anti-carbazole antibody coated microparticles and an anti-adamantane antibody/alkaline phosphatase conjugate (all of which are commercially available from Abbott Laboratories, Abbott Park, IL) were used in conjunction with the LCx® to capture and detect the reaction products. The average values from this experiment (calculated as counts/second/second; c/s/s) are presented in TABLE 2 and show the sensitivity of detection of different oncogenic HPV types to be approximately 10^3 to 10^4 molecules of DNA depending on the type.

TABLE 2

HPV Type	Molecules of HPV DNA (LCx® Rate: c/s/s)						
	0	10^3	10^4	10^5	10^6	10^7	10^8
HPV 16	96.7	307	1043.8	1266.5	1141.5	1001.7	993.4
HPV 18	100.6	1022.9	1345.2	1397.9	1361.4	1320.8	1256.8
HPV 31	99.0	262.8	970.0	1336.8	1344.2	1369.8	1282.7
HPV 33	103.8	280.9	896.2	963.2	846.0	665.4	654.6
HPV 35	100.5	303.9	1003.3	887.0	735.8	740.5	647.7
HPV 39	100.2	296.4	823.7	1009.3	899.5	733.4	640.5
HPV 45	94.2	429.7	923.7	1067.5	962.0	943.1	998.9
HPV 51	95.9	158.3	539.6	878.7	968.9	952.7	949.1
HPV 52	100.1	112.0	415.5	1146.4	1257.8	1167.9	1029.6
HPV 56	98.8	643.6	1100.8	1162.9	11244	1061.1	1157.2
HPV 58	104.4	306.3	1153.7	1463.3	1495.3	1520.6	1502.4
HPV 59	100.3	546.8	785.7	863.4	990.5	984.7	1014.8
HPV 68	86.4	279.1	349.6	1000.7	1011.9	899.5	877.7

Example 3

Specificity of HPV Detection

In addition to the 13 plasmids of oncogenic types of HPV obtained in Example 2, 12 plasmids of non-oncogenic types of HPV (HPV 6, 11, 13, 26, 32, 40, 42, 54, 55, 57, 61 and 66) were obtained.

DNA was purified from these plasmids as in Example 2 and diluted to 10^8 molecules of DNA/reaction. The HPV consensus primers (SEQ ID NOs. 1 and 2) and the HPV detection probes (SEQ ID NOs. 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37 and 40 for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, respectively) described in Example 1 were used to amplify and detect the diluted DNA samples as described above in Example 2 except that the detection probes were used at a final concentration of 4 nM each in separate reactions to detect the amplification products produced by the HPV consensus primers. The data from this experiment is presented in TABLE 3 and shows specific amplification and detection of oncogenic types of HPV only, with the non-oncogenic types of HPV being non-reactive.

TABLE 3

HPV Type	HPV Probe Type (LCx® Rate c/s/s)												
Onc	16	18	31	33	35	39	45	51	52	56	58	59	68
16	1350	-	-	-	-	-	-	-	-	-	-	-	-
18	-	1291	-	-	-	-	-	-	-	-	-	-	-
31	-	-	1479	-	-	-	-	-	-	-	-	-	-
33	-	-	-	836	-	-	-	-	-	-	-	-	-
35	-	-	-	-	1211	-	-	-	-	-	-	-	-
39	-	-	-	-	-	1039	-	-	-	-	-	-	-
45	-	-	-	-	-	-	1015	-	-	-	-	-	-
51	-	-	-	-	-	-	-	1016	-	-	-	-	-
52	-	-	-	-	-	-	-	-	1049	-	-	-	-
56	-	-	-	-	-	-	-	-	-	1282	-	-	-
58	-	-	-	258	-	-	-	-	-	-	1249	-	-
59	-	-	-	-	-	-	-	-	-	-	-	1296	-
68	-	-	-	-	-	-	-	-	-	-	-	-	979
Non-onc													
6	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-	-	-	-	-	-	-
61	-	-	-	-	-	-	-	-	-	-	-	-	-
66	-	-	-	-	-	-	-	-	-	-	-	-	nt

(Onc = oncogenic HPV types; Non-onc = non-oncogenic HPV types; A negative symbol (-) denotes tht the LCx® rate was less than 120 c/s/s; nt = not tested.)

The data in Table 3 shows that the oncogenic HPV probes specifically detect the HPV types for which they are designed to detect.

Example 4
HPV Detection in Clinical Samples

A. Detection of HPV in Clinical Samples Using the HPV LCx® Assay and a Commercial Hybrid Capture Assay Ninety-eight clinical samples were tested for oncogenic HPV by Digene's Hybrid Capture Assay (Digene Diagnostics, Silver Spring, MD) and compared to HPV detection using the HPV consensus primers (SEQ ID NOs. 1 and 2) and the HPV detection probes (SEQ ID NOs. 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37 and 40 for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, respectively) described in Example 1. Samples were collected in Digene sample buffer and processed according to the manufacturer's instructions. A portion of the sample was precipitated with ethanol at -70°C and the pellet resuspended in 50 µl of 10 mM Tris, 1 mM EDTA, pH 8.0. Ten µl of this sample was then added to 90 µl of 50 mM EPPS (N-[2-Hydroxyethyl]Piperzaine-N'-[3-Propane Sulfonic Acid], pH 8.0 (Sigma Chemical Co., St. Louis, MO), 14 mM $MgCl_2$ and heated to 95-100°C for 10 minutes. After cooling for 15 minutes to room temperature, sample was amplified and detected in a total reaction volume of 0.2 ml as in Example 2 (except $MgCl_2$ was added during sample preparation as stated above). Results are shown in Table 4.

TABLE 4

		HPV LCx® Assay	
		Positive	Negative
Digene Hybrid Capture Assay	Positive	28	2
	Negative	20	48

In Table 4 above, 28 of 98 samples were HPV positive by both assays and 48 of 98 samples were HPV negative by both assays. The 2 samples identified as positive by the Digene assay but negative in the HPV LCx® assay were confirmed as negative in an alternate PCR assay performed at Johns Hopkins University where the clinical samples were

obtained. Twenty samples were identified as HPV positive by the LCx® assay but were not detected by the Digene assay. Sixteen of these 20 samples were confirmed as positive in the assay at Johns Hopkins; the other 4 samples were retested as positive in the LCx® assay and typed as oncogenic using each probe in a separate reaction as in Example 3. Further proof of this was demonstrated by taking 14 of the Digene negative/LCx® positive samples, doing serial 10-fold dilutions from 1:10 to 1:1000 and testing these dilutions in the LCx® format. All 14 of these samples were detected at the highest 1:1000 dilution.

B. Detection of HPV in Biopsy Confirmed Cancer Specimens Thirty-eight samples were obtained from patients with biopsy confirmed cancer. These samples were tested for oncogenic HPV by Digene's Hybrid Capture Assay (Digene Diagnostics, Silver Spring, MD) and compared to HPV detection using the HPV consensus primers (SEQ ID NOs. 1 and 2) and the HPV detection probes (SEQ ID NOs. 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37 and 40 for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, respectively) described in Example 1. Samples were collected in Digene sample buffer and processed according to the manufacturers instructions. A portion of the sample was precipitated with ethanol at -70°C and the pellet resuspended in 50 µl of 10 mM Tris, 1 mM EDTA, pH 8.0. Three µl of the sample was diluted in 97 µl of 10 mM Tris, pH 8.0, 14 mM $MgCl_2$ and heated to 95-100°C for 10 minutes. After cooling for 15 minutes to room temperature, sample was amplified and detected in a total reaction volume of 0.2 ml as in Example 2 (except $MgCl_2$ was added during sample preparation as stated above). Results are shown in Table 5.

Table 5

		HPV LCx® Assay	
		Positive	Negative
Digene Hybrid Capture Assay	Positive	18	0
	Negative	15	5

Both assays detect oncogenic HPV in 18 of 38 cancer specimens and both are negative for 5 of the 38 samples. These 5 samples were

also found HPV negative by the Johns Hopkins alternate PCR assay. Fifteen of the cancer specimens were detected as containing oncogenic HPV by the LCx® assay but were negative in the Digene assay. However it should be noted that the Digene test does not include probes for 2 of the 15 LCx® positive/Digene negative subtypes detected (HPV types 39 and 58).

C. Detection of HPV in Biopsy Confirmed High Grade Cervical Interepithelial Neoplasia Specimens Twenty-two samples were obtained from patients with biopsy confirmed high grade cervical interepithelial neoplasia (H-CIN), prepared and tested as above in Example 4.B. comparing the HPV LCx® assay to Digene's Hybrid Capture Assay for detection of oncogenic HPV. Results are shown in Table 6.

Table 6

		HPV LCx® Assay	
		Positive	Negative
Digene Hybrid Capture Assay	Positive	10	0
	Negative	9	3

Both assays detect oncogenic HPV in 10 of 22 H-CIN specimens and both are negative for 3 of the 22 samples. These 3 samples were also found HPV negative by the Johns Hopkins alternate PCR assay. Nine of the H-CIN specimens were detected as containing oncogenic HPV by the LCx® assay but were negative in the Digene assay. It should be noted that of these 9 discrepant samples, 2 contained an HPV type (HPV type 58) which is not tested for in the Digene assay.

While the invention has been described in detail and with reference to specific embodiments, it will be apparent to one skilled in the art that various changes and modifications may be made to such embodiments without departing from the spirit and scope of the invention.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: NUCLEIC ACID SEQUENCES FOR DETECTING ONCOGENIC HUMAN PAPILLOMAVIRUSES

(iii) NUMBER OF SEQUENCES: 41

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Macintosh
(C) OPERATING SYSTEM: System 7.0.1
(D) SOFTWARE: Microsoft Word 5.1a

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAACAATGAC AACAACTATG ATG 23

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAAAAATAAA CTGTAAATCA TATTC 25

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 16)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTGTTACTG TTGTTGATAC TACACGCAGT ACAAATATGT CATTATGTGC 50
TGCCATATCT ACTTCAGAAA CTACATATAA AAATACTAAC TTTAAGGAGT 100
ACCTACGACA TGGGGAGGAA TATGATTTAC AGTTTATTTT TC 142

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCTGCCATAT CTACTTCA 18

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGAAGTAGAT ATGGCAGC 18

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 18)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTTGTTACTG TGGTAGATAC CACTCCCAGT ACCAATTTAA CAATATGTGC 50
TTCTACACAG TCTCCTGTAC CTGGGCAATA TGATGCTACC AAATTTAAGC 100
AGTATAGCAG ACATGTTGAG GAATATGATT TGCAGTTTAT TTTTC 145

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTAGCATCAT ATTGCC 16

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCAATATGA TGCTAC 16

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 31)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTGTTACTG TGGTAGATAC CACACGTAGT ACCAATATGT CTGTTTGTGC 50
TGCAATTGCA AACAGTGATA CTACATTTAA AAGTAGTAAT TTTAAAGAGT 100
ATTTAAGACA TGGTGAGGAA TTTGATTTAC AATTTATATT TC 142

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCAATTGCAA ACAGTGAT 18

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCACTGTTT GCAATTGC 18

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 33)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTTGTTACTG TGGTAGATAC CACTCGCAGT ACTAATATGA CTTTATGCAC 50
ACAAGTAACT AGTGACAGTA CATATAAAAA TGAAAATTTT AAAGAATATA 100
TAAGACATGT TGAAGAATAT GATCTACAGT TTGTTTTTC 139

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGCACACAA GTAACTAGT 19

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACTAGTTACT TGTGTGCAT 19

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 35)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTTGTTACTG TAGTTGATAC AACCCGTAGT ACAAATATGT CTGTGTGTTC 50
TGCTGTGTCT TCTAGTGACA GTACATATAA AAATGACAAT TTTAAGGAAT 100
ATTTAAGGCA TGGTGAAGAA TATGATTTAC AGTTTATTTT TC 142

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCTGTGTC TTCTAGTG 18

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CACTAGAAGA CACAGCAG 18

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 39)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTTCTTACTG TTGTGGACAC TACCCGTAGT ACCAACTTTA CATTATCTAC 50
CTCTATAGAG TCTTCCATAC CTTCTACATA TGATCCTTCT AAGTTTAAGG 100
AATATACCAG GCACGTGGAG GAGTATGATT TACAATTTAT ATTTC 145

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTCTATAGAG TCTTCCATAC C 21

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGTATGGAAG ACTCTATAGA G 21

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 45)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTTGTTACTG TAGTGGACAC TACCCGCAGT ACTAATTTAA CATTATGTGC 50
CTCTACACAA AATCCTGTGC CAAGTACATA TGACCCTACT AAGTTTAAGC 100
AGTATAGTAG ACATGTGGAG GAATATGATT TACAGTTTAT TTTTC 145

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTACACAAAA TCCTGTG 17

(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CACAGGATTT TGTGTAG

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 51)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TTTATTACCT GTGTTGATAC TACCAGAAGT ACAAATTTAA CTATTAGCAC 50
TGCCACTGCT GCGGTTTCCC CAACATTTAC TCCAAGTAAC TTTAAGCAAT 100
ATATTAGGCA TGGGGAAGAG TATGAATTGC AATTTATTTT TC 142

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGGTTTCCCC AACAT 15

(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATGTTGGGGA AACCG 15

(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 52)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TTTGTCACAG TTGTGGATAC CACTCGTAGC ACTAACATGA CTTTATGTGC 50
TGAGGTTAAA AAGGAAAGCA CATATAAAAA TGAAAATTTT AAGGAATACC 100
TTCGTCATGG CGAGGAATTT GATTTACAAT TTATTTTTC 139

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTGCTGAGGT TAAAAAG 17

(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTTTTTAACC TCAGCAC 17

(2) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 56)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTTGTTACTG TAGTAGATAC TACTAGAAGT ACTAACATGA CTATTAGTAC 50
TGCTACAGAA CAGTTAAGTA AATATGATGC ACGAAAAATT AATCAGTACC 100
TTAGACATGT GGAGGAATAT GAATTACAAT TTGTTTTTC 139

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTACAGAACA GTTAAGTAA 19

(2) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TTACTTAACT GTTCTGTAG 19

(2) INFORMATION FOR SEQ ID NO:33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 58)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTGTTACCG TGGTTGATAC CACTCGTAGC ACTAATATGA CATTATGCAC 50
TGAAGTAACT AAGGAAGGTA CATATAAAAA TGATAATTTT AAGGAATATG 100
TACGTCATGT TGAAGAATAT GACTTACAGT TTGTTTTTC 139

(2) INFORMATION FOR SEQ ID NO:34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AACTAAGGAA GGTACAT 17

(2) INFORMATION FOR SEQ ID NO:35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGTACCTTC CTTAGTT 17

(2) INFORMATION FOR SEQ ID NO:36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 59)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TTTTTAACAG TTGTAGATAC TACTCGCAGC ACCAATCTTT CTGTGTGTGC 50
TTCTACTACT TCTTCTATTC CTAATGTATA CACACCTACC AGTTTTAAAG 100
AATATGCCAG ACATGTGGAG GAATTTGATT TGCAGTTTAT ATTTC 145

(2) INFORMATION FOR SEQ ID NO:37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CTACTACTCT CTATTCCTAA TG 22

(2) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CATTAGGAAT AGAGAGTAGT AG 22

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (HPV type 68)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TTTCTTACTG TTGTGGATAC CACTCGCAGT ACCAATTTTA CTTTGTCTAC 50
TACTACTGAA TCAGCTGTAC CAAATATTTA TGATCCTAAT AAATTTAAGG 100
AATATATTAG GCATGTTGAG GAATATGATT TGCAATTTAT ATTTC 145

(2) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTTTGTCTAC TACTACTGA 19

(2) INFORMATION FOR SEQ ID NO:41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCAGTAGTAG TAGACAAAG 19

Claims

What is claimed is:

1. A oligonucleotide cocktail for detecting the presence of at least one oncogenic HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 in a test sample, said cocktail comprising at least two probes selected from the group consisting of: SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 40 and SEQ ID NO. 41.

2. The cocktail of claim 1 wherein one of said at least two probes is selected from the group consisting of: SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 40 and SEQ ID NO. 41.

3. A probe selected from the group consisting of: SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 40 and SEQ ID NO. 41.

4. A method of detecting the presence of an oncogenic HPV type in a test sample comprising the steps of:

a) contacting said test sample with at least one probe of claim 3; and

b) detecting hybridization between said probe and an oncogenic HPV type target sequence as an indication of the presence of an oncogenic HPV type in said test sample.

5. The method of claim 4 wherein said oligonucleotide is labeled.

6. The method of claim 4 wherein said test sample is contacted with a cocktail of probes comprising at least two probes.

7. The method of claim 6 wherein said at least two probes include SEQ ID NO. 37 and SEQ ID NO. 40

8. A method for detecting the presence of at least one oncogenic HPV type in a test sample comprising the steps of:

a) forming a reaction mixture comprising nucleic acid amplification reagents, at least one amplification primer for amplifying an oncogenic HPV target sequence, a test sample containing an oncogenic HPV type target sequence, and at least one probe according to claim 3; and

b) subjecting said mixture to hybridization conditions to generate at least one nucleic acid sequence complementary to said target sequence;

c) hybridizing said probe to said nucleic acid complementary to said target sequence so as to form a hybrid comprising said probe and said nucleic acid; and

d) detecting said hybrid as an indication of the presence of at least one oncogenic HPV type in said sample.

9. The method of claim 8 wherein said probe is labeled with a capture label and said primer is labeled with a detection label.

10. The method of claim 8 wherein said probe is labeled with a detection label and said primer is labeled with a capture label.

11. A kit comprising:

a) at least one probe according to claim 3, and

b) amplification reagents.

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(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
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25 June 1998 (25.06.98)

(54) Title: NUCLEIC ACID PRIMERS AND PROBES FOR DETECTING ONCOGENIC HUMAN PAPILLOMAVIRUSES

(57) Abstract

Probe sequences that are useful for detecting oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are herein provided. These sequences can be used in hybridization assays or amplification based assays designed to detect the presence of these oncogenic HPV types in a test sample. Additionally, the sequences can be provided as part of a kit.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19467

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 527 898 A (BAUER HEIDI M ET AL) 18 June 1996 see the whole document ---	1-11
X	WO 95 22626 A (STICHTING RES FONDS PATHOLOGIE ;MEIJER CHRISTOPHORUS JOANNES L (NL) 24 August 1995 see the whole document --- -/--	1-8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
1 April 1998	22. 04. 1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Knehr, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19467

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 90 01564 A (MICROPROBE CORP) 22 February 1990 see abstract see page 6, line 4 - line 25 see page 9, line 13 - line 26 see page 12, line 27 - page 13, line 9 see page 15, line 36 - page 16, line 12 see page 22, line 18 - page 23, line 35 see page 56, line 27 - page 57, line 38 see claims 24,36-39,46,52,66-68,74 see claims 79-81,84; table 4 ---	1,4-6, 8-11
Y	EP 0 489 442 A (SCLAVO SPA) 10 June 1992 see the whole document ---	1,4-6, 8-11
Y	MELCHERS W ET AL: "INCREASED DETECTION RATE OF HUMAN PAPILLOMAVIRUS IN CERVICAL SCRAPES BY THE POLYMERASE CHAIN REACTION AS COMPARED TO MODIFIED FISH AND SOUTHERN-BLOT ANALYSIS" JOURNAL OF MEDICAL VIROLOGY, vol. 27, no. 4, 1 April 1989, pages 329-335, XP000373982 see the whole document ---	1,4-6,8
Y	BERNARD C ET AL.: "Detection of human papillomavirus by in situ polymerase chain reaction in paraffin-embedded cervical biopsies." MOLECULAR AND CELLULAR PROBES, vol. 8, 1994, pages 337-343, XP002061048 see the whole document -----	1,4-6,8

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/19467

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1,3 (partial); 4-6,8-11 (complete)

Invention 1: Oligonucleotide probes (SEQ IDs NO:4,5) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 16 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

2. Claims: 1,3 (partial)

Invention 2: Oligonucleotide probes (SEQ IDs NO:7,8) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 18 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

3. Claims: 1,3 (partial)

Invention 3: Oligonucleotide probes (SEQ IDs NO:10,11) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 31 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

4. Claims: 1,3 (partial)

Invention 4: Oligonucleotide probes (SEQ IDs NO:13,14) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 33 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

5. Claims: 1,3 (partial)

Invention 5: Oligonucleotide probes (SEQ IDs NO:16,17) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 35 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

6. Claims: 1,3 (partial)

Invention 6: Oligonucleotide probes (SEQ IDs NO:19,20) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 39 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

7. Claims: 1,3 (partial)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 7: Oligonucleotide probes (SEQ IDs NO:22,23) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 45 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

8. Claims: 1,3 (partial)

Invention 8: Oligonucleotide probes (SEQ IDs NO:25,26) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 51 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

9. Claims: 1,3 (partial)

Invention 9: Oligonucleotide probes (SEQ IDs NO:28,29) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 52 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

10. Claims: 1,3 (partial)

Invention 10: Oligonucleotide probes (SEQ IDs NO:31,32) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 56 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

11. Claims: 1,3 (partial)

Invention 11: Oligonucleotide probes (SEQ IDs NO:34,35) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 58 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

12. Claims: 1-3,7 (partial)

Invention 12: Oligonucleotide probes (SEQ IDs NO:37,38) derived from the L1 region of the HPV genome for detection of the presence of oncogenic HPV type 59 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

13. Claims: 1-3,7 (partial)

Invention 13: Oligonucleotide probes (SEQ IDs NO:40,41) derived from the L1 region of the HPV genome for detection

of the presence of oncogenic HPV type 68 in a test sample by hybridization or amplification-based hybridization. Methods and a kit using them are included.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/19467

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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